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Diffusivity and structural polymorphism in some model stratum corneum lipid systems

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Mixtures of model stratum corneum lipids were prepared in water from cholesterol, six fatty acids and ceramides. The influence of composition on the polymorphism of these mixtures and also on the diffusivity of a model drug within them, D_{lip}, was determined. The former was obtained from X-ray diffraction and Fourier transform infrared spectrometry, and the latter from a diffusional release model. An L_β structure was formed for the composition approximating that of the extracellular lipids in intact human abdominal stratum corneum. D_{lip} was independent of water content in the range 20–40% w/w, with the bilayers showing one dimensional swelling without lateral expansion. Although removal of the ceramides did not result in a significant alteration in D_{lip}, crystalline cholesterol now appeared. The ceramides were, therefore, necessary for solubilization within the fatty acid bilayers of the large proportion of cholesterol present in the lipid fraction of intact SC. They were also responsible for a thermal L_α-H_{II} transition observed at approx. 68°. At the concentration in which it exists in intact SC, cholesterol also had only a minimal effect on D_{lip}, but was necessary to suppress H_{II} phase formation within the fatty acids and ensure an L_β structure. All lipid mixtures that had an L_β structure presented a diffusional barrier approx. 1 order of magnitude greater than that of an unstructured, isotropic lipid mixture. H_{II} structures formed at cholesterol/fatty acid proportions less than approx 8:92 mol% and appeared more permeable than L_β ones. All the results indicate that the diffusional barrier within the model lipid mixtures is guaranteed essentially by the presence of an L_β phase. Although the ceramides and cholesterol exert no intrinsic influence on the magnitude of D_{lip}, their presence in necessary for the existence of an L_β phase at 33° that is free of both crystalline cholesterol and H_{II} character.

Introduction

Human stratum corneum (SC) consists of several layers of keratinized corneocytes, surrounded by an ordered, extracellular lipid structure. The remarkably low permeability of SC is a consequence of two structural barriers existing within this heterogeneous membrane. A primary geometrical barrier is formed by the relatively impermeable corneocytes, and produces an extracellular diffusional pathway through the SC of extended path length and reduced area [1]. A secondary lipid barrier along this diffusional pathway is formed by a continuous system of extended bilayers between the corneocytes. The geometrical barrier can be described quantitatively by two-dimensional 'brick-and-mortar' models to represent the SC's heterogeneous internal structure. Although these describe accurately the importance of corneocyte dimensions and geometrical arrangement for the SC's permeability [2], they do not correctly predict well-established experimental findings concerning the extracellular lipid barrier. The rate of water loss across intact SC correlates, for example, better to its weight-percent lipid content than to either of the geometrical factors SC thickness or number of corneocyte layers [3]. This finding cannot be reproduced theoretically with any 'brick-and-mortar' model that does not take into account the complex, heterogeneous composition and structure of the extracellular lipid barrier. The lipid composition differs greatly from that of other biomembranes, being characterized by a virtual absence of phospholipids and enrichment with cholesterol, fatty acids and sphin-

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Abbreviations: SC, human stratum corneum; D_{lip}, drug diffusivity in model lipid mixture; ν_{CH}, frequency of symmetric CH_2-stretching vibration; ϕ_a, molar volume fraction of lipids; d, lamellar repeat distance; d_a, lipid-layer thickness; d_w water-layer thickness.

golipids, together with smaller quantities of triacylglycerols, sterol esters and hydrocarbons [4,5].

Our ultimate aim is to develop an improved biophysical model for the SC by incorporating not only its primary geometrical barrier, but also a quantitative description of its extracellular lipid barrier. In the present study we attempt to determine the basic relationship between the composition and barrier function of the extracellular lipid structure. This was characterized by measuring the diffusivity of a foreign substance (in our case, a drug of moderate lipophilicity) within mixtures of model SC lipids that form ordered structures in vitro [6] and whose composition can be varied at will. We conducted a systematic investigation of selected mixtures of the three major lipid components of the SC, viz fatty acids, ceramides and cholesterol. The dependence of drug diffusivity on lipid composition was determined. The structural polymorphism of the model SC lipid mixtures was examined using X-ray diffraction and Fourier transform infrared spectrometry. Clearly, the lipid mixtures do not reproduce some possibly important properties of the extracellular lipid barrier, e.g., the presence of the corneocyte lipid envelope or the existence of geometrical constraints within the narrow inter-corneocyte regions. Despite this limitation, we could obtain some insight into the basic barrier and structural properties of the unusual lipid combination peculiar to the SC.

Materials and Methods

Measurement of drug diffusivity in model SC lipid mixtures

The model SC lipids were used as received from Sigma Chemicals (Deisenhoven, Germany) with a stated purity of 99%. Each lipid mixture was prepared in water under N_2 by repeated heating to 80° with centrifugation through a 0.7 mm glass capillary, as developed by Friberg [6]. Sufficient NaOH was added to each mixture to neutralize 41% of the fatty acids and promote thereby the formation of an ordered structure [6]. The model drug tiamenidine (Hoechst, Frankfurt, Germany) of molecular weight 306 and pK_a 8.4 was incorporated into each mixture (0.2% w/w) during its preparation. The diffusivity of this drug in the lipid structure, D_{lip}, was determined at 33° ± 0.2° using a matrix release model we had developed earlier [2]. In brief, the release of drug from each lipid mixture was measured using a twin-compartment diffusion cell, in which the lipid mixture was separated from a stirred, aqueous acceptor phase by a porous PTFE membrane. The time-dependent rate of release was then fitted to the corresponding solution of the linear form of the diffusion equation for this problem under non-sink boundary conditions [7]. This yielded the best value for the space-independent D_{lip}.

Examination of polymorphism within the model SC lipid mixtures

The structural order existing within each lipid mixture was classified using X-ray diffraction at both 25° and 75°. The samples were examined in a glass capillary on an MAR Research maschine (40 kV) using Ni-filtered Cu-K_α radiation ($\lambda = 0.1542$ nm), a sample/MAC-image plate detector distance of either 400 cm (for small angle) or 160 cm (for wide angle), and an illumination time of 20 min. The X-ray diffraction patterns are given here as the dimensionless scattering intensity, $I(\theta)$, vs. the scattering vector, Q, in reciprocal nm. Q is given by $4\pi \sin \theta/\lambda$, in which θ is the scattering angle between the primary beam and scattered intensity.

Infrared spectra were obtained from the lipid mixtures using a Philips Model PU9800 Fourier transform infrared spectrophotometer equipped with a DTGS detector. The samples were treated at 80° for 15 min before being examined in a thermostatted Specac 20710 cell (Specac, Kent, UK) fitted with CaF_2 windows and using 25 μm Teflon spacers. Each spectrum was obtained by collecting 250 interferograms with a nominal resolution of 2 cm^{-1} and triangular apodization using the sample shuttle accessory. The spectrophotometer was continually purged with dry air to remove atmospheric water vapour. Samples were scanned between −6° and 75° at 2° intervals, with a 5 min delay between consecutive scans. Frequencies at the centres of gravity were determined by fitting the ten top points of each band to a Gaussian function using Spectra-Calc (Galactic Industries, Salem, USA).

Results and Discussion

The composition of the lipid fraction of intact, human, abdominal SC [4,5] is most closely approximated by mixture I (cf. Table I), which contained fatty acids, cholesterol and ceramides in the molar ratio

TABLE I

Lipid compositions of the model mixtures (mol%)

Lipid	Density * [g/ml]	I	II	III	IV
Fatty acids		46.6	100–68	100	100
stearic acid	0.941	9.39	9.39	19.5	13.7
palmitic acid	0.853	38.7	38.7	80.5	56.6
myristic acid	0.844	4.49	4.49	0	6.56
oleic acid	0.984	31.6	31.6	0	0
linoleic acid	0.869	12.0	12.0	0	17.6
palmitoleic acid	0.900	3.82	3.82	0	5.56
Cholesterol	1.052	26.2	0–32	0	0
Ceramides	0.90	27.4	0	0	0

* From: Friberg, S., Suhaimi, H., Goldsmith, L. and Rhein, L. (1988) J. Disp. Sci. Technol. 9, 371–389.

46.6 : 26.2 : 27.4. The small-angle X-ray diffraction pattern of this mixture at 25° showed a pure one-dimensional, lamellar periodicity for all water contents between 20–40% w/w. The example of 30% water is illustrated in Fig. 1a, with three reflections corresponding to the Bragg spacing ratios of $1:1/2:1/3$ [8]. The lamellar repeat distance, d, is larger with increasing water content and follows the relation $\log d = \log d_a - k \log \phi_a$ in the range 20–40% w/w water (Fig. 2a) seen with various neat phase structures [9] (ϕ_a is the total lipid molar volume fraction). The thickness of the lipid layer, $d_a = \phi_a d$, is 3.80 nm and independent of the water content in this range (Fig. 2b). Thus ideal, one-dimensional swelling of the lamellar structure occured, i.e., the water was only incorporated between and not within the lipid layers. Despite the presence of the fatty acid soaps, the lipid layers of fatty acids, cholesterol and ceramides have no detectable affinity for water. The slope $k = \mathrm{d} \log d/\mathrm{d}(-\log \phi_a)$ in Fig. 2a is precisely 1.0, confirming that ideal swelling occured [10] without lateral expansion of the bilayers. As intact SC hydrates without substantial change in the repeat distance of the lipid phase [11], the water must

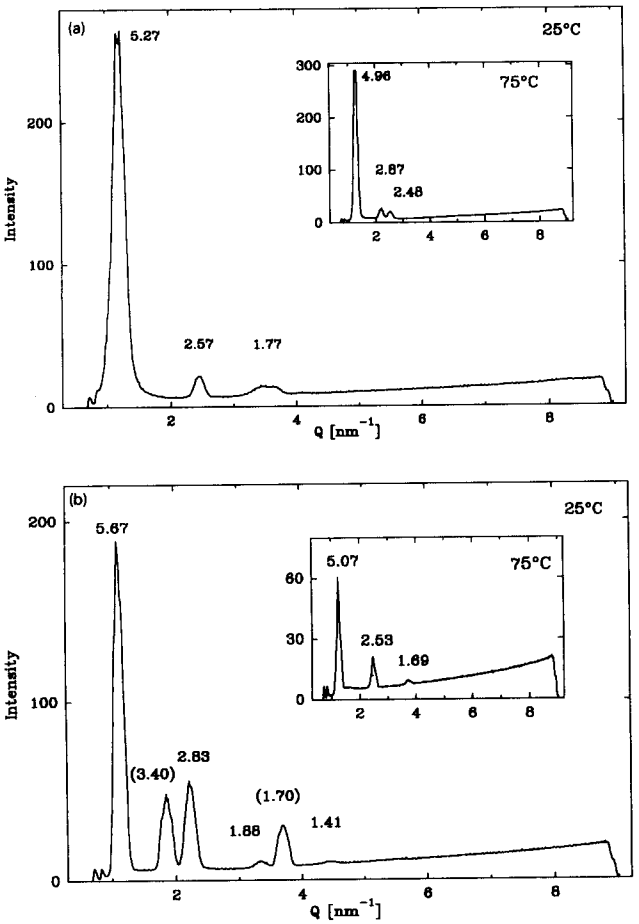

Fig. 1. Small-angle X-ray diffraction patterns. (a) Mixture I containing 30% w/w water; (b) mixture II (cholesterol/fatty acids 32:68) containing 30% w/w water.

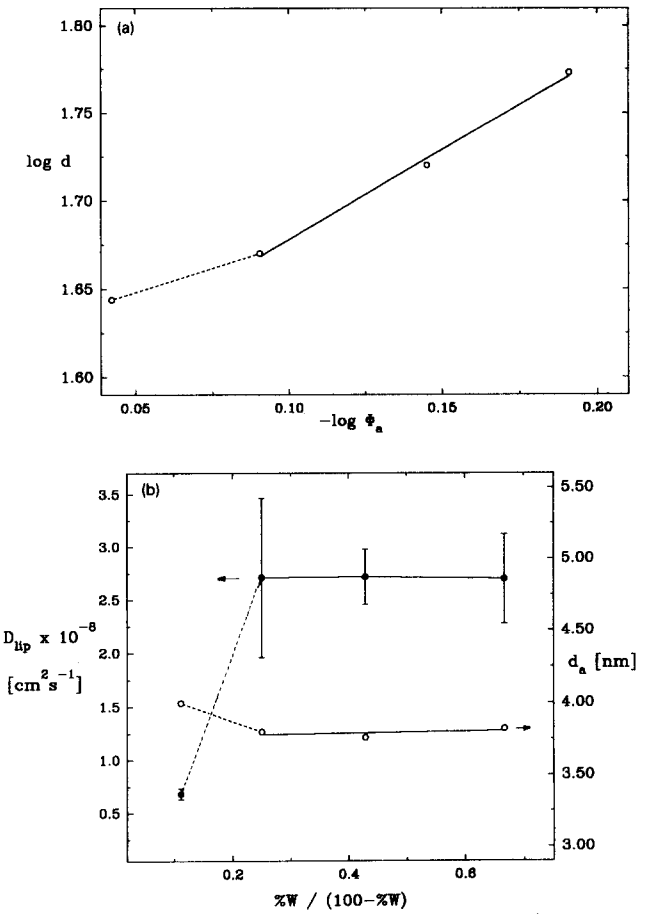

Fig. 2. Influence of water content on mixture I. (a) $\log d$ vs. $(-\log \phi_a)$; (b) d_a (○) and D_{lip} (●) vs. water content (%W/100-%W).

be taken up primarily into the corneocytes and not into the lipid phase. The equilibrium water content of the lipid fraction of intact SC is approx. 14% w/w [12]. At this water content, mixture I has the dimensions $d = 4.5$ nm and $d_w = 0.7$ nm (d_w is the thickness of the water-layer between two lipid-layers). This d compares well with the smallest of the three values determined for intact human SC, viz 4.4 [13], 6.5 and 13 nm [11].

The mixture I containing 10% w/w water formed a wax-like solid showing a drug diffusivity, D_{lip}, that is substantially smaller than those found at 20% w/w water (Fig. 2b). Although its small-angle X-ray diffraction pattern showed lamellar periodicity, the repeat distance, d, deviates from the logarithmic relation (Fig. 2a), since the thickness of its lipid-layer, d_a, is larger than that at higher water content (Fig. 2b). In the range 20–40% w/w water the continual increase in d (Fig. 2a) does not cause any change in D_{lip} (Fig. 2b). Fig. 1a shows that mixture I changes to a two-dimensional, hexagonal periodicity at 75°. The decrease in repeat distance is consistent with increased disorder of the fatty acids' acyl chains between a lamellar gel (L_β) and inverse hexagonal (H_{II}) state. This was confirmed in the wide angle X-ray region, where the three reflec-

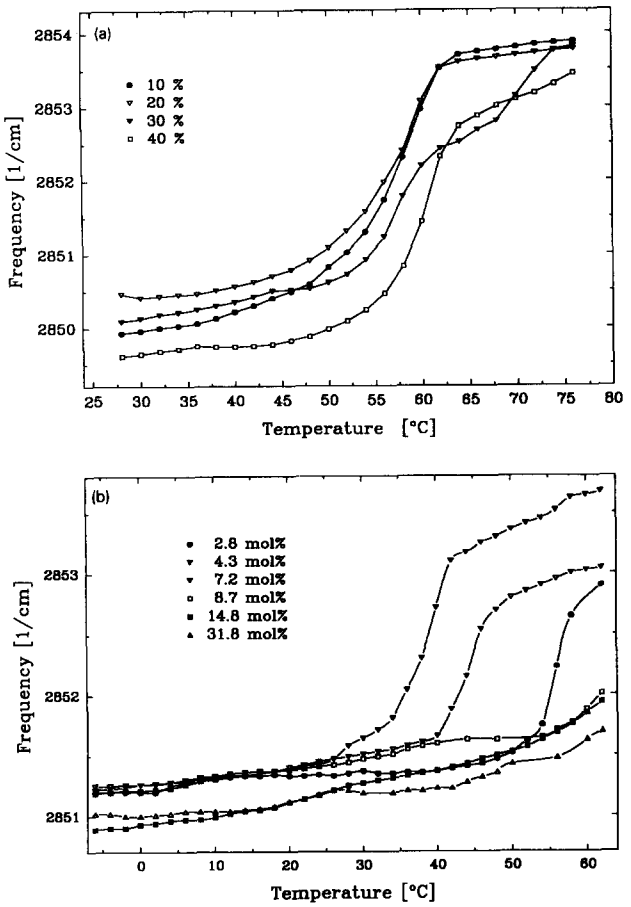

Fig. 3. Temperature-dependence of the symmetric CH_2-stretching vibration, ν_{CH}. (a) Influence of water content in mixture I; (b) influence of proportion cholesterol/fatty acid in mixture II.

tions at 0.42, 0.43, and 0.46 nm at 25° characteristic of acyl chains in the gel state were replaced at 75° by a diffuse halo stretching between 0.4 and 0.5 nm, characterstic of a fluid hydrocarbon core. Fig. 3a illustrates for each water content a shift in frequency of the symmetric CH_2-stretching vibration, ν_{CH}, of approx. 3 cm^{-1} between 55 and 75° that is characteristic of a transition from a gel–liquid crystalline state of the acyl chains. With 30% water, two separate transitions are evident, the first at 57 of approx. 2 cm^{-1} and the second at 68° of approx. 1 cm^{-1}. The identical behaviour seen for the acyl chains of phosphatidylethanolamines [14] comes from the first L_β-L_α transition with a subsequent L_α-H_{II} transition at higher temperature. These high transition temperatures reflect the large proportion of saturated to unsaturated fatty acids in mixture I (0.54 : 0.46). The fatty acids' acyl chains are, therefore, predominantly in the gel state at 33°, although the possibility of regional segregation of the saturated and unsaturated chains cannot be excluded. If the rate of diffusion through intact gel-phase bilayers is taken as negligible, D_{lip} is an orientational average for relaxation through the aqueous layers and

TABLE II

Measured drug diffusivities, D_{lip}, in selected lipid mixtures

Mixture	Structure	$D_{lip} \cdot 10^8$ [cm^2/s]
I	L_β	2.72 ± 0.26 [a]
II (32 mol% cholesterol)	L_β	1.61 ± 0.12 [b]
III	isotropic	27.4 ± 2.20
IV	L_β	2.44 ± 0.97

[a] From Fig. 2b;
[b] From Fig. 4a.

around the barrier-like lipid-layers of an extended lamellar structure. The extent of partitioning of the drug molecules from the aqueous into the lipid-layers remains to be established and may be of crucial importance for diffusion through bilayer structures. For the present case, however, the combined changes in diffusional path length and area of the aqueous layers with increasing water content in mixture I are insufficient to cause a measureable alteration in D_{lip} (cf. Fig. 2b).

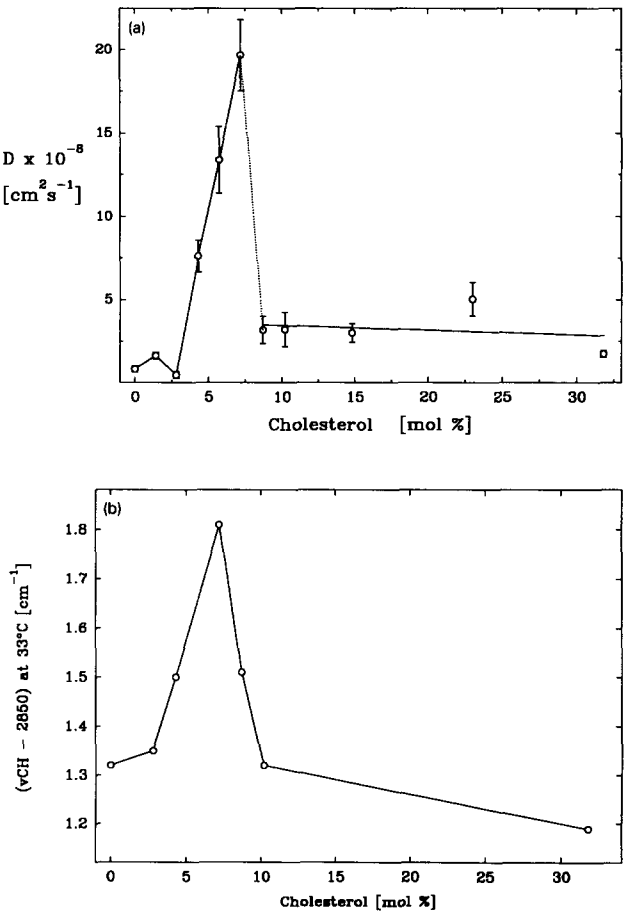

Fig. 4. (a) Dependence of drug diffusivity, (D_{lip}), on cholesterol content of fatty acid mixture II; (b) change in frequency of the symmetric CH_2-stretching vibration of the cholesterol/fatty acid mixtures II, ν_{CH}, measured at 33°C.

Of the three lipid components of mixture I, only the fatty acid/soaps form a mesophase when mixed alone with water [6]. It was not possible to prepare isolated mesophases from either ceramides [6] or cholesterol [15] alone. The importance of ceramides for the barrier function of mixture I could, however, be deduced by removing it to yield mixture II (cholesterol/fatty acids 32:68, in Table I). This resulted in a slight decrease in D_{lip} from $2.7 \cdot 10^{-8}$ cm^2/s to $1.5 \cdot 10^{-8}$ cm^2/s (Table II). Removal of the ceramides still leaves a lamellar structure at 25° (Fig. 1b), but two additional reflections are seen at 3.4 and 1.7 nm, characteristic of hydrated, crystalline cholesterol. The ceramides in mixture I are, therefore, necessary for the solubilization of cholesterol within the bilayers, but do not contribute substantially to the barrier function. The L_β–L_α transition in mixture II up to 75° (Fig. 1b) is characterized by the observed reduction in lamellar repeat distance, but there is no subsequent transition to an H_{II} structure. The temperature of this transition lies above 60°, as seen from the frequency of the symmetric CH_2-stretching vibration at this proportion of cholesterol/fatty acids (Fig. 3b). The absence of a lamellar to H_{II} transition up to 80° has also been found with a ceramide-free mixture of hydrated palmitic acid, cholesterol and cholesterol sulphate [16]. The ceramides in mixture I are, therefore, responsible for its thermal L_α to H_{II} transition.

The influence of cholesterol on the barrier function of mixture II is illustrated in Fig. 4a. In the range 0–2.8 mol% cholesterol in the fatty acids there is no change in D_{lip} and the small angle X-ray diffraction patterns show a mixed structure (Fig. 5a). A lamellar periodicity of 4.36 nm with corresponding reflections at 2.17 (1/2), 1.45 (1/3), and 0.87 (1/4) nm dominates a hexagonal perodicity of 5.37 nm with reflections at 3.10 ($1/\sqrt{3}$), and 2.69 (1/2) nm. As the latter is an H_{II} phase, the possibility of regional segregation of the saturated and unsaturated fatty acids cannot be excluded. At 75° a transition to a pure H_{II} phase is complete (Fig. 5a),

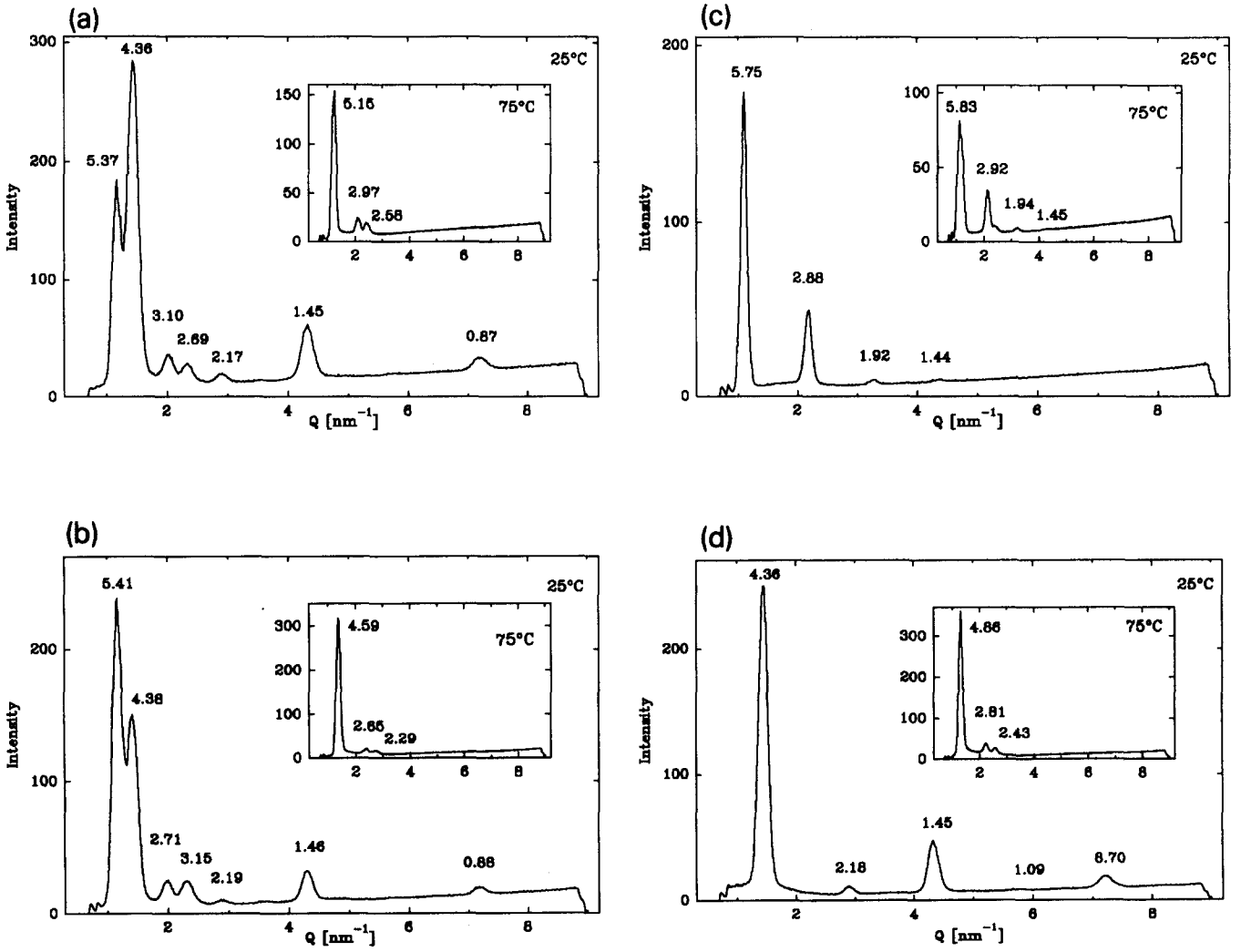

Fig. 5. Small-angle X-ray diffraction patterns (all containing 30% w/w water). (a) Mixture II with 0 mol % cholesterol; (b) mixture II with 8.7 mol% cholesterol; (c) mixture II with 23 mol% cholesterol; (d) mixture IV.

whose repeat distance lies intermediate between the lamellar and H_{II} phases existing at 25°C. The striking, 20-fold increase in D_{lip} between 2.8 and 7.2 mol% cholesterol (Fig. 5a) is accompanied by a parallel increase in the frequency of the symmetric CH_2-stretching vibration at 33° (Fig. 4b). The lamellar component of the mixed fatty acid structure is, therefore, an L_β phase, which is fluidized by the cholesterol in this concentration range, as also seen, for example, with phosphatidylcholines [14]. As confirmation of this, more than 2.8 mol% cholesterol in the fatty acids progressively increased the intensity of the X-ray hexagonal reflections at the expense of the lamellar ones, until with 8.7 mol% cholesterol (Fig. 5b) the hexagonal periodicity dominates. The inverted truncated cone-shaped cholesterol molecule promotes an isothermal $L\beta$-H_{II} transition [17]. The fluidization of the fatty acids' acyl chains associated with this transition evidently results in the sharp increase in D_{lip} occuring between 2.8 and 7.2 mol% cholesterol (cf. Fig. 4a). This isothermal fluidization is also evident from the thermal transition to a pure H_{II} state that occurs at approx. 55° for both 0 and 2.8 mol% cholesterol, but which is reduced to below 35° with 7.2 mol% cholesterol (Fig. 3b).

With more than 7.2 mol% cholesterol, the previously-dominating H_{II} phase suddenly disappears, leaving a pure lamellar structure as shown in Fig. 5c for the example of 23 mol% cholesterol. A major restructuring of the lipids has occured, since the lamellar repeat distance is now notably larger (5.8 nm) than that of the L_β phase existing at lower cholesterol concentrations (5.4 nm). High concentrations of cholesterol eliminate $L\beta$–$L\alpha$ transitions [14], and indeed the lamellar phase shows no transition up to 75° (see inset). The decrease in ν_{CH} at 33° between 7.2 and 10 mol% cholesterol (Fig. 4b) together with the sharp jump in transition temperature to over 60° (Fig. 3b) confirms condensation of the mixed L_β/H_{II} to a pure L phase in this range of cholesterol concentration. This results in the precipitous fall in D_{lip} seen in Fig. 4a at this point. With further increase in cholesterol concentration D_{lip} is unchanged. The ratio of cholesterol to the fatty acids in mixture I is 32:68 (mol%). We conclude that at this concentration the contribution of cholesterol to the barrier function of mixture II is that of suppressing the formation of H_{II} phase by the unsaturated components of the fatty acids (cf. Fig. 5a), ensuring thereby an L_β structure.

It has been suggested that the existence of a bilayer structure per se is sufficient to account for the barrier function in lipid mixtures [18]. More insight into this effect can be reached as follows. The two major saturated fatty acids found in the SC, stearic and palmitic acids, formed only an isotropic mixture alone in water (mixture III in Table I), since the Krafft temperature of these lipids lies well above 25°. In the absence of any mesophase structure, the measured D_{lip} is ten times larger than that for the L_β structure of mixture I (Table II) that contained all six fatty acids, cholesterol and ceramides. The addition of the remaining fatty acids, except oleic acid, to mixture III (to form mixture IV) results in a lamellar structure at 25° with a transition to H_{II} by 75° (Fig. 5d). D_{lip} decreased to the same value as that for mixture I (Table II). The primary barrier of the lipid mixtures comes, therefore, from the L_β structure and not from particular lipid components.

Conclusions

Although lipid composition influences the barrier function of the lipid mixtures, the values of D_{lip} are all of the order of 10^{-8} cm^2/s. These are 1–2 orders of magnitude smaller than diffusivities for drugs of similar molecular weight in isotropic semi-solids such as silicones or hydrogels [19]. Neither cholesterol nor ceramide, when present in the proportions in which they exist within intact SC, assume intrinsic importance for determining the magnitude of D_{lip}. The ceramides are necessary for solubilization of cholesterol within bilayers formed by the fatty acids, and are also responsible for the thermal L_α-H_{II} transition observed for mixture I at approx. 68°. Cholesterol suppresses the formation of an H_{II} phase by the unsaturated fatty acids in mixture II, promoting thereby an L_β structure. All lipid mixtures having an L_β structure demonstrated diffusivities approx. one order of magnitude larger than those in a non-ordered lipid mixture. The isothermal transition of an L_β to an H_{II} phase caused by the addition of up to 7.8 mol% cholesterol to the six fatty acids in the absence of ceramides, produced a parallel increase in D_{lip}. We conclude that the H_{II} phase was more permeable than the L_β one as a result of increased acyl chain mobility. We stress, however, that the possible influence of equilibrium partitioning of the drug molecules from the aqueous into the lipid-layers on D_{lip} remains to be clarified and may be an important factor. This is currently being investigated and will be reported in due course. According to the results presented here, however, the typical variation in fatty acid/cholesterol/ceramide composition of intact SC for different body sites [4] would, for example, alter D_{lip} only within the range of approx. $1.0–3.0 \cdot 10^{-8}$ cm^2/s. This difference is insufficient to account for the observed differences in permeability of these body sites [20], even when the respective lipid contents and SC thicknesses are included in the calculation (Lieckfeldt and Lee, unpublished results).

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